

*Supplementary Information for*

**An adenylyl cyclase with a phosphodiesterase domain in basal plants  
with a motile sperm system**

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**This contains Supplementary Tables S1-S3 and Figure S1-S4.**

**Table S1 Effect of bicarbonate on adenylyl cyclase activity of GST-MpCAPE-AC**

| Proteins  | Specific activity (pmol min <sup>-1</sup> mg <sup>-1</sup> ) <sup>a,b</sup> |
|---|---|
| GST-MpCAPE-AC (Mg <sup>2+</sup> )                                       | 0.39 ± 0.05   |
| GST-MpCAPE-AC (Mg <sup>2+</sup> , 50 mM HCO <sub>3</sub> <sup>-</sup> ) | 0.35 ± 0.04   |

<sup>a</sup>Mean values ± standard deviation (*n* = 4)

<sup>b</sup>Adenylyl cyclase activities were assayed as described in Methods except for using 200 mM Tris-HCl (pH 7.5) instead of 50 mM Tris-HCl (pH 7.5). One mM MgCl<sub>2</sub> and 50 mM NaHCO<sub>3</sub> were used.

**Table S2 cAMP phosphodiesterase activity of His-MpCAPE-PDE proteins**

| Proteins             | Phosphodiesterase activity (pmol min <sup>-1</sup> ) <sup>a,b</sup> |
|----------------------|---|
| His-MpCAPE-PDE       | 573 ± 19  |
| His-MpCAPE-PDE-H199Q | n.d. <sup>c</sup>   |
| His-MpCAPE-PDE-H203Q | n.d. <sup>c</sup>   |

<sup>a</sup>Mean values ± standard deviation (*n* = 3)

<sup>b</sup>cAMP phosphodiesterase activities were assayed as described in Methods with MgCl<sub>2</sub>.

<sup>c</sup>n.d. = not detected

**Table S3 Accession number of registered adenylyl cyclase genes in phylogenetic tree**

| Species                           | Abbreviation               | Accession No.                 |
|-----------------------------------|----------------------------|-------------------------------|
| <i>Viridiplantae and Euglena</i>  |                            |                               |
| <i>Marchantia polymorpha</i>      | <i>Marchantia</i>          | Mapoly0068s0004 <sup>*1</sup> |
| <i>Physcomitrella patens</i>      | <i>Physcomitrella</i>      | Pp3c8_21740 <sup>*1</sup>     |
| <i>Selaginella moellendorffii</i> | <i>Selaginella</i>         | 427736 <sup>*1, 2</sup>       |
| <i>Adiantum capillus-veneris</i>  | <i>Adiantum</i>            | LC164752                      |
| <i>Coleochaete orbicularis</i>    | <i>Coleochaete</i>         | GBSL01008739.1 <sup>*3</sup>  |
| <i>Chara braunii</i>              | <i>Chara</i>               | LC169160                      |
| <i>Klebsormidium flaccidum</i>    | <i>Klebsormidium</i>       | kfl00481_0040 <sup>*4</sup>   |
| <i>Mesostigma viride</i>          | <i>Mesostigma</i>          | GBSK01038777.1 <sup>*5</sup>  |
| <i>Chlamydomonas reinhardtii</i>  | <i>Chlamydomonas</i>       | EDP05013                      |
| <i>Ostreococcus tauri</i>         | <i>Ostreococcus</i>        | XM_003079447                  |
| <i>Cocomyxxa subellipsoidea</i>   | <i>Cocomyxxa</i>           | XM_005649187                  |
| <i>Micromonas pusilla</i>         | <i>Micromonas</i>          | XM_003056838                  |
| <i>Euglena gracilis</i>           | <i>Euglena</i> PACa-C1     | AM181334                      |
|                                   | <i>Euglena</i> PACa-C2     | AM181334                      |
| <i>Alveolata</i>                  |                            |                               |
| <i>Plasmodium falciparum</i>      | <i>Plasmodium</i>          | AJ289136                      |
| <i>Fungi</i>                      |                            |                               |
| <i>Saccharomyces cerevisiae</i>   | <i>Saccharomyces</i> CYR1  | M12057                        |
| <i>Neurospora crassa</i>          | <i>Neurospora</i>          | XM_011394894                  |
| <i>Bacteria and Cyanobacteria</i> |                            |                               |
| <i>Sinorhizobium meliloti</i>     | <i>Shinorhizobium</i> CyaA | M35096                        |
| <i>Arthrosphaera platensis</i>    | <i>Arthrosphaera</i> CyaC  | D49692                        |
|                                   | <i>Arthrosphaera</i> CyaG  | D49531                        |
| <i>Mammalia</i>                   |                            |                               |
| <i>Rattus norvegicus</i>          | Rat type2-C1               | AAA40682                      |
|                                   | Rat type2-C2               | AAA40682                      |
| <i>Bos taurus</i>                 | Bovine type1-C1            | NP_776654                     |
|                                   | Bovine type1-C2            | NP_776654                     |
| <i>Homo sapiens</i>               | Human type10(sAC)-C1       | NP_060887                     |
|                                   | Human type10(sAC)-C2       | NP_060887                     |

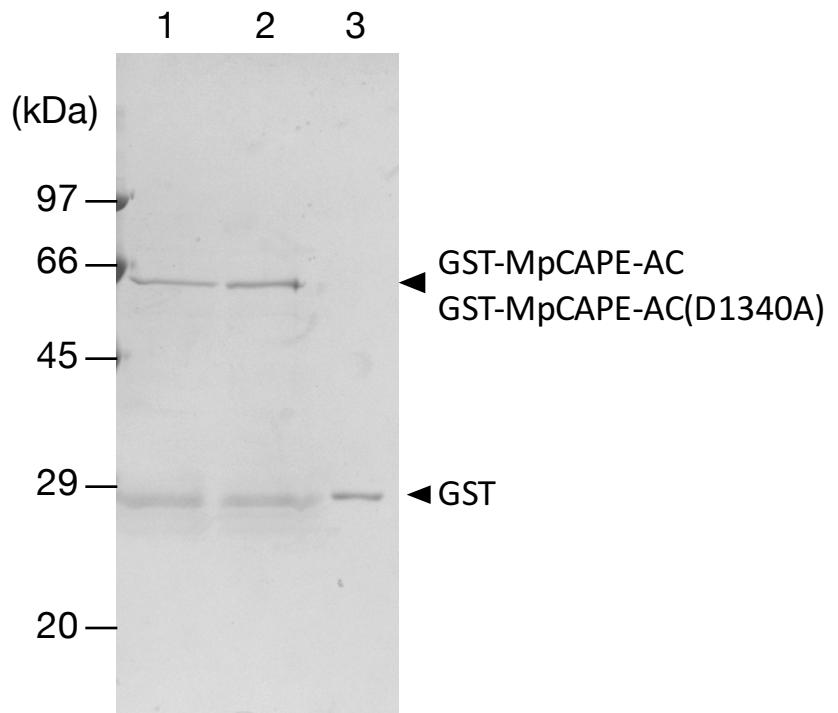
<sup>\*1</sup><https://phytozome.jgi.doe.gov><sup>\*2</sup>The cDNA sequence in this study was reconstituted from the predicted ORF sequence (427736) and the genome DNA sequence to obtain the best match to the sequence from *Marchantia polymorpha*.<sup>\*3</sup>NCBI TSA accession number GBSL01000000 (Ju et al. *Nat. Plants*, **1**, 14004 (2015))<sup>\*4</sup>[http://www.plantmorphogenesis.bio.titech.ac.jp/~algae\\_genome\\_project/klebsormidium/](http://www.plantmorphogenesis.bio.titech.ac.jp/~algae_genome_project/klebsormidium/)<sup>\*5</sup>NCBI TSA accession number GBSK01000000 (Ju et al. *Nat. Plants*, **1**, 14004 (2015))

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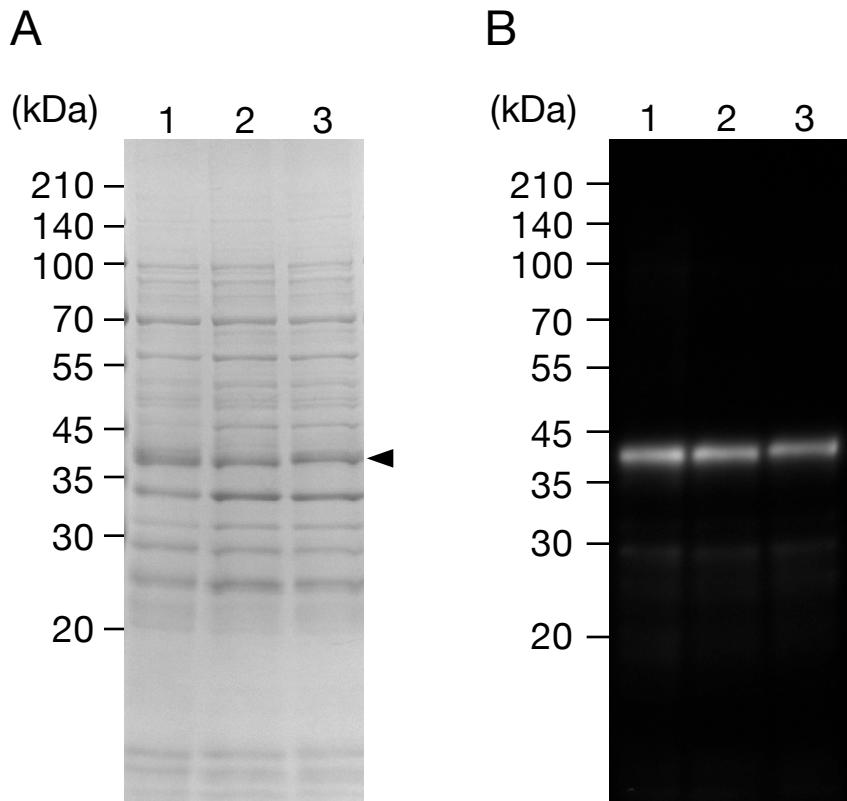
1 MEKPELKDDKGKDQEENDEAKNKRPRDLIRIPLLNLERAREYVPPASPTEK
51 TASDSEMLIISELPNIGQPDFSFSPVLEREDSPKLKAPSALSDEAVQQVL
101 QGINSWTFDIFQLDDSLPKMVEKIFRELGLFDNFPLDVKKVRAFTNAMEV
151 MRYQPNPYHNFRHACDVLHAVYLILTLVDGRKKLSHLEVFAALALAALCHD
201 VDHPGLTNAFLVATYDPLALRYNDRAVLESHAATCFITMRGNDSLNL
251 GLSEEQRHMRKLMIVLILATDMGEHARILREVGERVQDLRPFEQSPFYT
301 PPGCLSPILRDAESSSSGNTTAGAKSSDAPPSPKRLPDVKYKNPLSPSPP
351 IQSTSDVMILLIQLIIKCADISNVVKPFFLSKRWAALLLWEWFRQGEIEKQ
401 LGLPISKFMDREDPSTMAMTCGCIDYIAKPMYEVTMKLLPRMHENVLVN
451 LNLRQMWSTFSTNGRRASETAQQILGPFAPPPIPKGEAVQEGYQQDHSK
501 LEGKLVATFSGQYVSSDVPSRKLESSPSAKSLGSVSEDSEETVENVEDSP
551 ILSVTDRSSEFSRSLGSEAGSSVKTSPQFLSRAGTEAPSSPQSPLQASSEQ
601 RGSPSPGVQYRLSPRGPGSPSPVQTQISGNPESPGYRETYGPGGSQPAAM
651 EVPFVQKSPHEPVAVDVDAASQQSADVIAPLSKPQALQVKQPVVPTVSR
701 GVHFDDSELTASERSELSSSIQSGSSPDALRVSFVAINPDSDSVKVLIE
751 QSDPLSTATIEQRDPDSPRMTGLEPFLEEVAASSSESKAEPETEDEYNQR
801 YEAPLSLLFGAPTREELMLPAKIGKDKNASLVSALLPGANNLVNDRKQNA
851 WEQMAFRSPSEQAASRGSLTTVPSFANSQDGDEKSSLRKGPPGFWEA
901 LRTHPRVNRLNRALESKTNALLIVATLVALFADDVVKGFLPKHADVYES
951 HILTACLSLFLAESALLCIFDRYFFSFFFWLDMLGSVSLVPLVIGITAQ
1001 NLDVIARTGRAAKTVTRFSKTLQASHIQQQIVHHIPVLRVFKFFGFKRDST
1051 LESPDYEEEKFLSKPSQLWSRSLAELTSQKLILGVLIIMILTPYFNNSEK
1101 DLAPLVSLDPLDDYLIGSPNFNLTVERVINMTKRHGYNLLYLGVKASCRS
1151 IKEGGYSYCLGIDVAGVEKYQQILPNVDEEPDGRQAAQEEFRPTELISVT
1201 SDSSRAQAYYSIKKSRFKYGMINIAMTVLILLLAACFFLSRDSNRLLI
1251 QPIERMVQFVKELAEDPVSFAGKTVVKPTGDSSKIMETFYVEAALVKIAS
1301 LTKVALGDAGMDILSVNLKGSEFNPMPLPGKKIRACFGFCDIRNFTDATEC
1351 LQEDVMMFVNRIADVHNKVVLHSGFPNKNIGDAFLIVWKKTVSDNTNKS
1401 RATSFADRALRAFLDIIQSIETSQSLAEFAKHPAIQKRMPGYRIHMGFGL
1451 HVGWAIEGAIGSAHKVDPSPYLSPHVNMASRLEATKQYGVMLLISETVIA
1501 HLTNSTLRDSCRKLDRTVKGSQDPMVLYTFDIPLFQQDLRGNPQEYRDI
1551 FEEAVDSYIDGDWDIALERLQECQTLWPTDKPATVLLTFMASHNNIAPEN
1601 WAGFRELTEK

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**Figure S1. Amino acid sequence of MpCAPE.** The catalytic domains of adenylyl cyclase (AC) and phosphodiesterase (PDE) are indicated by red (1335-1521) and green (106-452), respectively. Two membrane-spanning regions (TM1: 974-997 and TM2: 1220-1242) are indicated by blue. The membrane-spanning regions were predicted by a web site, TMHMM (<http://www.cbs.dtu.dk/services/TMHMM/>).



**Figure S2. Purification of GST-MpCAPE-AC protein.** Each affinity-purified protein (1.5 µg) was loaded onto a 12% SDS-PAGE gel for electrophoresis. The gel was stained with Coomassie Brilliant Blue. Lane 1, GST-MpCAPE-AC; lane 2, GST-MpCAPE-AC(D1340A); lane 3, GST. The positions of size markers are shown at the left side of the gel.



**Figure S3. Partial purification and immunodetection of His-MpCAPE-PDE proteins.** (A) Partially purified protein samples (10 µg) were loaded onto a 12% SDS-PAGE gel for electrophoresis. The gel was stained with Coomassie Brilliant Blue. The arrow head indicates the position of the His-MpCAPE-PDE proteins. The positions of size markers are shown at the left side of the gel. Lane 1: His-MpCAPE-PDE, lane 2: His-MpCAPE-PDE-H199Q, lane 3: His-MpCAPE-PDE-H203Q. (B) The partially purified protein samples (0.1 µg) were loaded onto a 12% SDS-PAGE gel for electrophoresis and subjected to immunoblotting with an anti-T7 epitope tag antibody for detection of His-MpCAPE-PDE proteins. The positions of size markers are shown at the left side of the gel. Lane 1: His-MpCAPE-PDE, lane 2: His-MpCAPE-PDE-H199Q, lane 3: His-MpCAPE-PDE-H203Q.

|        |      |   |      |
|--------|------|---|------|
| MpCAPE | 1    | M-----EKPELKDDKGKDQEENDEAKNKRPRDLIRIPLL-----  | 77   |
| PpCAPE | 1    | M-----  | 20   |
| SmCAPE | 1    | M-----EHKRPSIIRISKPRHSSLIQEEVLAALKVDVGR-----  | 34   |
| CbCAPE | 1    | MSRNASAGSWSRMVANPLSKTSKTPRGDTDEVGENPKPQRSGTSGPQDMADWSAKGLGHLGGSGKGLGVSGKDADDDELARAPPFESPRSHDAESRAA  | 100  |
| CoCAPE | 1    | SKTPELGRALTSSVAKASAALLKEAHDLQG-----   | 30   |
| MpCAPE | 78   | EREDSPKLKAPSALSDEAVQQV[LOG]NSWTFDTQLDDDSLPKMVEKFIRELGLFDNFPLDVKVKRAFTN--AMVMRYQPNPYHNFRACDVHLAVYLI  | 174  |
| PpCAPE | 21   | --HKS[LOG]ESWSFDFQVEDDQPLMVEKIFRS[N]NNFPIDM[H]KFRAFVR--AMVARQPNPYHCRACDVHLAVYLI   | 98   |
| SmCAPE | 35   | HELEGWLQEDLSWSDF[DI]FKVATEDLPKMA[RI]FVNGLLLTLPLDVKQLKA[ECVGMMAQRAHYPFHNRACDVHLAVYLI   | 118  |
| AcCAPE | 1    | PKMVERIFHD[G]FEALPLASKF[G]FVN--SIMQYRAHPYHNFRACDVHLAVYLI  | 57   |
| CbCAPE | 101  | VIQAFLLEEQNVIYDTSKSISIELLED[DSWE]FDFLVKEEIDLNRVVEHVCERLGLDTF[G]IDRNAFTIFVFS--HIQSQYOSNAYHNFRACDVHLAVYLI   | 197  |
| CoCAPE | 31   | --GIPLLTEEDIEVV[LQD]DGLSFDI[FR]IREEDHLK[VRT]FQRLGLEEFPIRISRV[RA]FVH--AIEQYQPNTYHNFRACDVHLAVYLI  | 118  |
| MpCAPE | 175  | LTLIVDGRKKL[SH]LEVFPLAALCHD[VDPGLTNAFLVATYDPLA]RYNDRALESHHAAT[FT]MRGNDSLNL[AGLSEE]EQHRMRKLMIVLILATDMG   | 274  |
| PpCAPE | 99   | LT[LAEG]AQRNQMEIFAV[AL]A[CHD[VDPGLTNAFLV]AASDPI]SRYNDRALESHHAAT[FT]MRGNDNVIM[SLDF]PQRHLR[LR]LVSCLILATDMG  | 198  |
| SmCAPE | 119  | LKL[G]ADEKFENQE[LK]LALCIS[ALCHD[VDPGLTNS]V]ACNPLA[R]YNDLSVLE[HH]ASITVAKLTGYESMIN[G]IFPDPS[EQRH]IRKLIV[CS]CLATDMA                                      | 218  |
| AcCAPE | 58   | LTV[EG]QKMTLEVFALLAALCHD[VDPGLTNS]SITNDPLA[R]YNDLSILESHHASTTIK[TL]AYESTNT[AP]SEA[EQRH]IRKLIV[E]ILATDMA  | 157  |
| CbCAPE | 198  | LT[OTE]GDL[SS]LEKVAII[S]ALCHD[VDPGLTNS]FLITNDPLA[R]YNDRSV[LES]HSAT[FR]VILDRKNATAIETFSKE[KALIRKLIV[G]TILATDMA  | 297  |
| CoCAPE | 119  | LT[OTE]AGPLLTSLEKLA[AM]GALCHD[VDPGLTNAFLV]AKPLA[R]YNDFALESHHAAT[F]K[Q]ERVEVD[G]Q[SEG]PRRH[LR]KLIV[IL]ATDMA  | 218  |
| MpCAPE | 275  | EHARILREVG[ER]VQDLRPFQ-----   | 321  |
| PpCAPE | 199  | VHAEIISSTGRLNDRRPFEL-----   | 247  |
| SmCAPE | 219  | YHGA[VQ]FSLSRK[EVGS]FHA-----  | 265  |
| AcCAPE | 158  | HHF[KI]I[ML]KSRS[SD]SN[P]FEC-----   | 202  |
| CbCAPE | 298  | Q[EDVL]GALQARLVDTKPFOV-----   | 346  |
| CoCAPE | 219  | [Q]SETVAAFAARMSDPRF[QT]VAPPPTPPQAAAAPAPAVTPRTPQLKIPTSRAASLRSPRPS[PH]ARTSHSSDATSQASTSKVHTDEGGHSRSE   | 318  |
| MpCAPE | 322  | -----AGAKS[D]AP-----SPKRLPD[KV]KNPLSPS[P]-----IOTS[V]MLI  | 361  |
| PpCAPE | 248  | -----DEAMSS[A]AP[PN]DNTNQPPP[ELK]SPNPKYLL[P]-----LTSSD[V]MLY  | 293  |
| SmCAPE | 266  | -----EQEE[E]A-----LLFPQVTKE-----HRSDD[V]LLM   | 292  |
| AcCAPE | 203  | -----QS[SG]S-----TTVPSR[D]-----PSST[K]DATTLL  | 229  |
| CbCAPE | 347  | -----LPKSDP[L]S-----VQTGTEFGERQETFYQSSATE[D]AILL  | 384  |
| CoCAPE | 319  | IQTQDAPSERERSSKGESIPSSRKGSQPPDKGVPRTSRVSISRTQSVTSAAVKG[AK]TPRESRASNNFSQSPPATT[V]P[N]PAHOTT[ST]DALLM   | 418  |
| MpCAPE | 362  | QLI[IK]CADISNV[K]PFPLSKRWAALLLEWFROGEIEKQLGPISKFMDRDPS[TL]M[TC]G[DI]YIAKPMYEWTM[KL]LPRMHEN[V]LN[LN]NRQMWSF  | 461  |
| PpCAPE | 294  | QMP[IK]CAD[SNV]KPFPLSKRWS[ALL]LEWFROGDIEREL[G]RVS[K]FEMR[RS]PTTLM[DT]G[DI]FV[AP]M[FE]ATAV[L]P[L]H[DE]ALAN[L]T[LR]LWSSF                                | 393  |
| SmCAPE | 293  | KVIM[K]CSD[DISN]TIRPYPLSK[KA]ALLLEWFROGDIE[ER]L[G]RVS[K]FEMR[RS]PTTLM[DT]G[DI]FV[AP]M[FE]ATAV[L]P[L]H[DE]ALAN[L]T[LR]LWSSF                            | 392  |
| AcCAPE | 230  | KM[IK]CADISNV[K]PFPLSKRWAALLLEWFROGDIE[ER]L[G]RVS[K]FEMR[RS]PTTLM[DT]G[DI]FV[AP]M[FE]ATAV[L]P[L]H[DE]ALAN[L]T[LR]LWSSF                                | 329  |
| CbCAPE | 385  | KM[IK]CADISNV[K]PFPLSKRWAALLLEWFROGDIE[ER]L[G]RVS[K]FEMR[RS]PTTLM[DT]G[DI]FV[AP]M[FE]ATAV[L]P[L]H[DE]ALAN[L]T[LR]LWSSF                                | 484  |
| CoCAPE | 419  | RMI[IK]CADISNL[IK]PFPLSKRWAALLLEWFQGDIEKRLGPISKFMDRDPS[TL]M[TC]G[DI]FV[AP]M[FE]ATAV[L]P[L]H[DE]ALAN[L]T[LR]LWSSF                                      | 518  |
| MpCAPE | 462  | STNGRRASETAQQILOP[F]APPIPK-----   | 535  |
| PpCAPE | 394  | SMGGRGSEVARNILOP[F]PPPI-----  | 442  |
| SmCAPE | 393  | STAGP[IE]QEVQV[IL]QSF[F]-----   | 431  |
| AcCAPE | 330  | TSNGS-[ALV]TLEKFLNSFN-----  | 368  |
| CbCAPE | 485  | TYRGRGSQEA[AL]I[EL]QHLDPLPSKSFQPRPEIERIKRRPLASAA[TP]MGGAYHLDPRVTE[QT]PMLQFQAGGSVRLIEWSSNLPHTDDPSHQATP   | 584  |
| CoCAPE | 519  | STNGRRGSATA[AD]ILOP[F]YP-----   | 557  |
| MpCAPE | 536  | SEDSEETVENVEDSP[LS]VTDRSSEFSRLGSEAGSSVKTSPQFLSRA[G]TEAPSS[PS]Q[LS]QASQE[RG]SPSPGVQYRLSPRGSPSPVPTQISGNPESPG  | 635  |
| PpCAPE | 443  | -----MYLP[G]VEKESL[P]APLQL-----   | 484  |
| SmCAPE | 432  | -----QTGSTMPES-----   | 460  |
| AcCAPE | 369  | -----Q[R]HVTY[GE]-----  | 400  |
| CbCAPE | 585  | DQVDDSDGGDLEVLRRAG-----GLPAIAEGSEHGKGVSKEVSSGS[DAKNI]SPLQKAIEIFPSSKGGMASLQRPSL[SDAGT]VIASSPLQPLRP   | 681  |
| CoCAPE | 558  | -----SELDSL[CAT]PAGG-----   | 596  |
| MpCAPE | 636  | YRETYPGGGSQPAAME-----V[P]FVQKSPHEPVAVDVDAASQOSADVIAPLSKQ[AL]QVQPVVPT[V]P[G]VHFDD[EL]TASERSELSSSIQSGS  | 725  |
| PpCAPE | 485  | LLALDSSGDKSQSINK-----GPI[R]-----DRESSI[G]G-AAPLS-----   | 535  |
| SmCAPE | 461  | -HTSPGNERPLSLLFG-----AP[IGR]-----DVNTLASE[AW]-----NTL[RD]-----SSVNLVPTRPSR-----   | 514  |
| AcCAPE | 401  | LETGKRTDPLSLLV[G]-----GPEEE-----NSSTS[RP]R[SG]-----SKIVDRSVOSSWDSSM[FD]RSQVSKFR-----  | 463  |
| CbCAPE | 682  | HGPSSA[GH]AATSA[SN]QFCPSV[ST]VGS[WSRC]ACCRAEANEIDPDQGGSKETWDMSMVNSMSTNENTEGKRGFAELVERIR-DISFRVRDIA  | 780  |
| CoCAPE | 597  | ITDQGRHHTASAVARG-----MIMRS-----NSQ[LA]E[SE]HRS[GE]KD-----   | 650  |
| MpCAPE | 726  | SPD[L]ALRVSFVAINPDSVSKV[LI]EQSDPLSTATIEQRDPDSRPM[TL]GLEP[FL]EEVAASSSES[KA]EPETEDEY[N]QRYEAPSL[LL]FGAPTREELMLPAIGK                                     | 825  |
| PpCAPE | 536  | GNN[L]AL-[SF]VALRNN-----  | 579  |
| SmCAPE | 515  | GSIGSLQ---LGPGQ-----  | 546  |
| AcCAPE | 464  | TTK[L]AFQPSKLH[G]K-----   | 517  |
| CbCAPE | 781  | ITH[LA]GEVMAEMNEDEISTIYSSNTSFTGA[KS]ASTAAM[VS]LFSKPD[NS]RPEGISSKHKKE[LP]VTAALAP[VE]SRTKAAGGPLTVAPESQVVASPIQ   | 880  |
| CoCAPE | 651  | SRLGFD[TL]AELNQGR-----LLADG-----  | 686  |
| MpCAPE | 826  | DKN[AS]VSALLPGANNVDRKQNAWEQMAFRSPSEQA[RS]GSLT[LL]V[TC]G[DI]FV[AP]M[FE]ATAV[L]P[L]H[DE]ALAN[L]T[LR]LWSSF   | 924  |
| PpCAPE | 580  | -KNAV[DI]Q[LS]FIQD[SA]WDRN[RC]-----   | 629  |
| SmCAPE | 547  | RHETS[LS]DRIDV[DK]GPVG-----   | 594  |
| AcCAPE | 518  | KHNP[S]---IQIASHEIS-----  | 561  |
| CbCAPE | 881  | KWSA[LT]TSNQVR[LS]SQVSSQMT-----   | 944  |
| CoCAPE | 687  | NNRPSHKMMSFRNIGRPT-----   | 733  |
| MpCAPE | 925  | VAT[VA]LFADDVVKGF[LP]KADYYE[SH]ILT[AC]L[AE]S[AL]C[IF]RDRY[F]FFF[FL]MLGSV[LP]V[LG]V[IG]T[VA]Q[N]L[VI]ART[RA]AKT[V]T[RF]SKT[LO]AS                       | 1024 |
| PpCAPE | 630  | IAT[IV]MFANDFTKA[LL]PKAG-----   | 701  |
| SmCAPE | 595  | PAT[LI]AL[FL]NDF[SR]AFL[PK]SLD[TF]FV[DV]V[VI]C[LF]L[LD]V[AL]S[VL]K[PA]Y[LS]F[EW]-----   | 676  |
| AcCAPE | 562  | PA[V]VIAL[FL]SHNFIRAYC[PA]ADP[Y]L[I]YLV[CL]A[FL]F[EL]V[AL]S[IV]R[KD]F[LG]MS[FW]L[DV]G[V]S[LP]I[LP]I[LG]MLE[IN]L[IM]ARA[Q]A[KS]V[RA]A[MM]QT            | 661  |
| CbCAPE | 945  | VCT[LV]GLFADD[Q]ALF[PK]EYD[KV]VRGAD[F]CFF[LS]F[DL]IA[C]IVRK[G]YF[FS]FFF[FL]DAVAT[IS]L[LP]V[IT]D[F]MK[N]L[SI]A[RA]G[RA]A[R]G[TR]A[R]L[RF]V             | 1044 |
| CoCAPE | 734  | IAT[FC]S[LS]F[DI]T[IA]L[PK]S[W]D[PA]E[T]Y[IL]V[CL]C[FILE]S[IL]S[VF]Q[V]G[Y]F[FS]FFF[FL]D[MI]A[TL]V[WP]D[IT]G[LIST]NL[VI]A[RA]G[RA]A[R]G[TR]A[R]L[RF]V | 833  |
| MpCAPE | 1025 | H[Q]QQIV[H]I[P]VLRVFKFFG-FKRDSTL[ESP]YEEEK-----   | 1118 |
| PpCAPE | 702  | PLSS---MFP[Y]I[L]KVFH-YKNVVDDDE[Y]FHV-----  | 791  |
| SmCAPE | 677  | SAP[K]VSA[FL]ARLAKAVFVHC[IR]PL[ES]KARD[KE]TTEDDS[NO]FMS[SK]P[YL]N[RL]S[DL]S[Q]R[VL]G[IL]I[LA]H[AL]P[Y]F[Q]L[P]D[LP]A[PL]L[AA]D[SP]L[Y]                | 776  |
| AcCAPE | 662  | DFSKVII[STA]LK[F]KRY---KVQ[TE]E[AN]K[DI]E[PD]P[DE]---   | 754  |
| CbCAPE | 1045 | RMAR-L[QL]L[TK]VRA[RR]GLFRRRRSDDDGDKDQSS---VIDS[SK]P[SV]W[RL]S[EL]T[Q]K[FL]I[G]V[AM]M[AB]PL[L]H[KD]L[V]N[LS]S[PL]L[H]S[PL]Y                           | 1140 |
| CoCAPE | 834  | R[LA]R---V[V]N[IG]R[TH]S[WL]W[QP]---H[K]GRM[DA]V[HE]F[DE]V[DT]-----   | 922  |

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|        |      |   |                              |      |
|--------|------|---|------------------------------|------|
| MpCAPE | 1119 | PNFNU[RE]TVRVINMTKRHGYNLLYLGVK-----   | -----                        | 1145 |
| PpCAPE | 792  | AHFNETIIDRIQT[F]NMLHGYNLLYLG[IN]-----   | -----                        | 818  |
| SmCAPE | 777  | LRFNFSI[L]NLNFNNOHDYAI[I]YL[GCR]-----   | -----                        | 803  |
| AcCAPE | 755  | AHFNETLQYLYDFYQSHGYNLLYLGVK-----  | -----                        | 781  |
| CbCAPE | 1141 | E[EF]NSTL[K]IMILE[N]REQQYDLYI[G]CNIIMKEVSSTGV[DIF]STNDNNNNNANGKRSLLSAFPGDIHMALQVVTNASDAPSAMGSTSIMGSEEK                                | -----                        | 1240 |
| CoCAPE | 923  | QVFNRTEELTAFENTFKFN[Y]K[V]YL[GVR]-----  | -----                        | 949  |
| MpCAPE | 1145 | -----   | -----                        | 1145 |
| PpCAPE | 818  | -----   | -----                        | 818  |
| SmCAPE | 803  | -----   | -----                        | 803  |
| AcCAPE | 781  | -----   | -----                        | 781  |
| CbCAPE | 1241 | NQRRLLLDTSAHPVEYNSPEDGGAGTDKPPENTLEKRLHVQLNP[GTA]KADAYSWPQSIAVPDLSHGEDGDQRGEELSAALTARWAKEPMFLKNPVRSLM                                 | -----                        | 1340 |
| CoCAPE | 949  | -----   | -----                        | 949  |
| MpCAPE | 1146 | -----ASCRSIIKEGGYSYCLGIDVAGV-----   | -EKYQJIL[P]NVDEEPDRQ         | 1185 |
| PpCAPE | 819  | -----GMCGW-----CALDSA-----  | -TNFMIVP-----SKGADGKK        | 847  |
| SmCAPE | 804  | -----DGQAQG-----YC[SG]YE[FG]SEG-----  | -DPFIQVVPPIEAGR[IA]KS        | 837  |
| AcCAPE | 782  | -----DE[GT]PS-----YVSSGECTSTQAGSMVCS-----   | -GLDSGRSTFIOLYPPSTKVA[D]GRK  | 831  |
| CbCAPE | 1341 | NSPLPELA[D]SDSNSVVGAGLGRHLAGALPLDEN[P]ASTLRSNNDI[R]LFSKQEGHDSKSP[P]A[SL]V[P]ARFA[S]FVPTCEERGK[ER]OYV[P]DMLQPE[G]KK                    | -----                        | 1440 |
| CoCAPE | 950  | -----VPDPPSHYQGNL[G]CYCTNSL-----  | -FDYDAV[P]VVLV[P]KLSDV[P]NGR | 995  |
| MpCAPE | 1186 | AAQEEFR-PTELISVTS[DS]SRAQAYYSIKKKSRFKYGMNIA-----M[VI]L[LI]AAWCFFLSDSNRLL-----IQPIERMVQFVKELAEDPVFSAGK                                 | -----                        | 1273 |
| PpCAPE | 848  | EAQEYR-TVELKSVLSDSNRTEALY[ST]RSELRTKH[AL]NA-----M[VI]LW[LI]GAWSFVLSNDSNRL-----IQPIERMV[E]IKV[EL]SDDPVFSFAAR                           | -----                        | 935  |
| SmCAPE | 838  | DVTKSYR-DEELIKVKS[D]SRRCEAFY[S]IRKRSRKHNVLNLET[LG]GADGAD[V]AVFVH[V]CFV[P]FKLGYOOFIGSGAFQPIERMVNF[K]ELA[D]NPGAFV[GK]                   | -----                        | 936  |
| AcCAPE | 832  | EAEAMYR-PTELIFIAS[FS]KCI[II]QRGQIYDMG-----A[VI]L[LI]SAWSFVLSNDSNRL-----IQPIERMVRFVKELAEDPVFSAGK                                       | -----                        | 919  |
| CbCAPE | 1441 | NGYEDFR-LLELSHFQT[G]G[SE]LFSEASRPNLQQA[AN]V-----LTLFVVAL[G]WGSFV[P]FDSNRL-----IQPIERMVFFF[K]ELAED[P]VFSAGK                            | -----                        | 1528 |
| CoCAPE | 996  | AVKKKFREP[K]ELLYAT[S]IAK[CE]AYD[SI]SAYSKKD[AW]NIT-----LTLFVVLV[LA]TWSFLLSDSNRL-----IQPIERMVFSV[K]ELA[D]DPVFSV[GK]                     | -----                        | 1084 |
| MpCAPE | 1274 | T[TV]VKPTGDSSK-----I[M]ETFY[VE]AALV[K]IASLT[K]VALGDAGMDILSVNLKG-----SEFNPM[LP]GKKIRACFGFCIDRNFTDATECLQEDVMMFVN[RI]ADVV                | -----                        | 1366 |
| PpCAPE | 936  | ANVKQPA[SS]VG-----QM[ME]TRV[VE]AALV[K]IASL[S]KVALGDAGMDILSVNLKG-----SEFNPM[LP]GKKIRACFGFCIDRNFTDATECLQEDVMMFVN[KI]ADVV                | -----                        | 1031 |
| SmCAPE | 937  | SISRKSRGKSG-----KV[ME]TRM[LE]GALV[K]IASL[S]KVALGDAGMDILSVNLKG-----A[E]FN-----RKKIRACFGFCIDRNFTDATECLQEDVMMFVN[KI]ADVV                 | -----                        | 1026 |
| AcCAPE | 920  | I[LP]PTKGGEG-----KM[ME]TRV[VE]AALI[K]IA[LT]KVALGDAGMDILSVNLKG-----TAFNPM[LP]GKKIRACFGFCIDRNFTDATECLQEDVMMFVN[KI]ADLV                  | -----                        | 1012 |
| CbCAPE | 1529 | IIGEHHEQGHV-----METSMVEGALMKIASLT[K]VALG[B]AG-----DILS[EN]LKG-----SEFNPL[LP]GKKIRACFGFCIDRNFTD[TE]CL[K]EVMMFVN[IA]QV[EN]              | -----                        | 1620 |
| CoCAPE | 1085 | SSTGDANFSEIRGLDQLM[ET]SV[EL]ALLKIASLT[K]V[LG]AG-----DILS[EN]LKG-----SEFNPM[LP]GKKIRACFGFCIDRNFTD[TE]CL[K]EVMMFVN[KI]AEVV              | -----                        | 1183 |
| MpCAPE | 1367 | H[HK]VVF[H]SG[FP]KNKIGDAFL-----IWKVKT[SD]NTNKS[RA]T-----SFADRALRAFLDIIQ[SI]ETSQS[LA]E[A]KHPAIQ[K]RMP[G]YR[IK]MGFGLHV[GWA]             | -----                        | 1455 |
| PpCAPE | 1032 | H[HK]VVF[H]SG[FP]KNKIGDAFL-----IWKVKSAA[D]NIQ[K]TRG[T]N-----SFADRALQSFLDIIQ[CI]ETSQ[LA]EYAH[HP]AIQ[K]RMP[G]YR[IK]MGFGLHV[GWA]         | -----                        | 1121 |
| SmCAPE | 1027 | H[HK]V[MI]HHG[FP]KNV[K]GDAFL-----IWKVKTMSDGAQ[K]SKGS-----SFADRALISFLDIIQ[AL]E[SE]V[HE]YAH[HP]AIQ[K]RMP[G]YR[IK]MGFGLHV[GWA]           | -----                        | 1115 |
| AcCAPE | 1013 | H[HK]VVIYH[G]Y[GP]NKNIGDAFL-----IWKVTT[H]MRGFGGG[S]APV[P]KGPGMS-----SFADRALQFLDIIY[S]VET[Q]O[Y]AH[HP]AIQ[R]RMP[G]YR[IR]L[G]FGLHV[GWA] | -----                        | 1101 |
| CbCAPE | 1621 | H[SRV]V[F]HGP[SP]NKNIGDAFL-----IWKVTT[H]MRGFGGG[S]APV[P]KGPGMS-----SFADRALKAFLDIIQ[DI]ETS[KS]LAQ[S]Q[H]PSI[Q]ARMP[G]F[K]IRLGFLHV[GWA] | -----                        | 1720 |
| CoCAPE | 1184 | H[HK]VVMH[G]SP[PN]KNIGDAFL-----IWKVKSISD[S]AT[K]TGT-----SFADRALRSFLD[Q]A[V]ES[Q]T[LA]EYAH[HP]AIQ[K]RMP[G]YR[IK]MGFGLHV[GWA]           | -----                        | 1272 |
| MpCAPE | 1456 | IIEGAIGSAH[K]VDP[SP]LSPHVN[MA]SRL[E]AAT[K]QYGV[ML]ISET[TV]IA[LT]KS[TD]RDS[CR]KLDR[V]TKGS[QD]PMVLYTFD[PL]FOODLR[CP]Q[PY]RDFEEAV        | -----                        | 1555 |
| PpCAPE | 1122 | IIEGAIGSAH[K]VDP[TP]YLSPHVN[VS]RLEAAT[K]QYGV[WM]ISET[TV]AEL[TK]SSLR[H]C[CR]KLDR[V]TKGS[AA]P[IT]TYF[D]PLFQ[Q]DLK[G]NPA[D]YR[AL]FENAV   | -----                        | 1221 |
| SmCAPE | 1116 | IIEGAIGSSH[K]VDP[SP]LSPHVN[MA]SRL[E]AAT[K]QYGV[WM]ISET[TV]AEL[TK]SSLR[H]C[CR]KLDR[V]TKGS[AD]PL[TT]YD[L]PFQ[Q]DLK[G]NPA[D]YR[AL]FENAV  | -----                        | 1215 |
| AcCAPE | 1102 | IIEGAIGSSH[K]VDP[TP]YLSPHVN[VS]RLEAAT[K]QYGV[WL]ISET[V]S[NT]LKSSLR[H]C[CR]KLDR[V]TKGS[EP]M[LY]TYD[L]PFQ[Q]DLV[EN]YABYKNTFEEAV         | -----                        | 1201 |
| CbCAPE | 1721 | IIEGAIGSSH[K]VDP[TP]YLSPHVN[VS]RLEAAT[K]QYGV[WL]ISET[V]S[NT]LKSSLR[H]C[CR]KLDR[V]TKGS[NE]PLS[LY]TCNT[IL]PQ[Q]DLG[T]T[D]YTRKFESGV      | -----                        | 1820 |
| CoCAPE | 1273 | IIEGAIGSSH[K]VDP[SP]LSPHVN[MA]SRL[E]AAT[K]QYGV[WL]ISET[TV]DNLNTK[TS]LRG[CR]KLDR[V]TKGS[AH]P[LS]L[FT]YD[PM]Q[RD]LKEP[Q]YKERFEEAV       | -----                        | 1372 |
| MpCAPE | 1556 | [D]SYI[D]G[D]D[IA]LERL[Q]EC[Q]TLWPTDKPATV[LT]IFTMASHNNIAPE[N]WA[FR]EL[TE]K  | -----                        | 1610 |
| PpCAPE | 1222 | DNYI[GN]NSA[LE]KU[EE]CQLWLP[TD]KPG[GH]VLLNFMASHNYRIPASW[K]YREL[TE]K   | -----                        | 1276 |
| SmCAPE | 1216 | DSYI[GN]HTA[LE]KLU[Q]WLP[TD]KPG[GH]VLLNFMASHNYRIPASW[K]YREL[TE]K  | -----                        | 1270 |
| AcCAPE | 1202 | DDYI[Q]GK[HE]ALD[K]LHW[Q]HLP[TD]KPEDV[IA]FMASH[K]NVP[ON]W[K]YREL[KE]  | -----                        | 1256 |
| CbCAPE | 1821 | EAYINGNWEATEYLED[K]ALWPS[DK]PAQVLLA[FM]ATYNNQP[D]NRCFREL[TE]K   | -----                        | 1875 |
| CoCAPE | 1373 | NAYIAGD[W]AV[A]VEKL[KE]Q[K]IMP[TD]KPAQVLLN[MS]LGNRSP[H]WQ[FR]EL[TE]K  | -----                        | 1427 |

**Figure S4. Alignment of MpCAPE with its orthologues.** MpCAPE amino acid sequence is aligned with the orthologous sequences from *Physcomitrella patens* (PpCAPE), *Selaginella moellendorffii* (SmCAPE), *Adiantum capillus-veneris* (AcCAPE), *Coleochaete orbicularis* (CoCAPE) and *Chara braunii* (CbCAPE). Amino acid residues identical in majority of sequences are shown in black boxes. Gap introduced for good alignment are indicated by dashes. Number are amino acid positions for each protein sequence. Note that AcCAPE is a partial sequence. The catalytic domains of adenylyl cyclase (AC) and phosphodiesterase (PDE) are indicated by red and green bar, respectively.